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A Longitudinal Study of Antimicrobial Resistance in Enterococcus spp. Isolated from a Beef Processing Plant and Retail Ground Beef — Source link

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1 **A Longitudinal Study of Antimicrobial Resistance in *Enterococcus* spp. Isolated from a**
2 **Beef Processing Plant and Retail Ground Beef**

3

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16 **Running title:** Antimicrobial resistance of *Enterococcus* spp.

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24 **ABSTRACT**

25 Antimicrobial use in food-producing animals has come under increasing scrutiny due to its
26 potential association with antimicrobial resistance (AMR). Monitoring of AMR in indicator
27 microorganisms such as *Enterococcus* spp. in meat production facilities and retail meat products
28 can provide important information on the dynamics and prevalence of AMR in these
29 environments. In this study, swabs or samples were obtained from various locations in a
30 commercial beef packing operation (n = 600 total) and from retail ground beef (n = 60) over a
31 19-month period. All samples/swabs were enriched for *Enterococcus* spp. and suspected
32 enterococci isolates were identified using species-specific PCR primers. *Enterococcus faecalis*
33 was the most frequently isolated species followed by *Enterococcus hirae,* which was found
34 mostly on hides and ground beef. *Enterococcus faecium* (n = 9) and *E. faecalis* (n = 120)
35 isolates were further characterized for antimicrobial resistance and resistant genes due to the
36 clinical significance of these species. Twenty-one unique AMR profiles were identified, with
37 90% of isolates resistant to at least two antimicrobials, and two that were resistant to nine
38 antimicrobials. Tetracycline resistance was observed most often in *E. faecalis* (28.8%) and was
39 likely mediated by *tet*(M). Genomic analysis of selected *E. faecalis* and *E. faecium* isolates
40 revealed that many of the isolates in this study clustered with other publicly available genomes
41 from ground beef, suggesting that these strains are well adapted to the beef packaging
42 environment.

43

44 **IMPORTANCE**

45 Antimicrobial resistance (AMR) is a serious challenge facing the agricultural industry.
46 Understanding the flow of antimicrobial resistant-bacteria through the beef fabrication process

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47 and into ground beef is an important step in identifying intervention points for reducing AMR. In
48 this study we used enterococci as indicator bacteria for monitoring AMR in a commercial beef
49 packaging facility and in retail ground beef over a 19-month period. Although washing of
50 carcasses post-hide removal reduced the isolation frequency of *Enterococcus* spp., a number of
51 antimicrobial resistant-*Enterococcus faecalis* isolates were recovered from ground beef produced
52 in the packaging plant. Genome analysis showed that several *E. faecalis* isolates were genetically
53 similar to publicly available isolates recovered from retail ground beef in the United States.
54

55 **INTRODUCTION**

56 *Enterococcus* spp. are often used as indicators of fecal contamination due to their
57 association with the mammalian gastrointestinal tract and persistence in the environment (1). The
58 concentration of enterococci in the feces of cattle varies, but is typically around 10^4 to 10^5 CFU
59 g^{-1} (2, 3). Previous studies have reported that *Enterococcus* spp. are prevalent in ground beef
60 samples in North America (4-7) but less information is available regarding the prevalence of
61 enterococci in the beef processing environment.

62 Presently, there are more than 60 species of *Enterococcus* and two subspecies (LPSN;
63 http://www.bacterio.net) with *Enterococcus faecalis* and *Enterococcus faecium* most frequently
64 associated with ground beef (4, 5). Certain strains of these species are also responsible for
65 serious nosocomial infections and vancomycin-resistant enterococci (VRE) strains are
66 particularly difficult to treat (8, 9). Many enterococci are intrinsically resistant to several
67 antimicrobials and also acquire resistance through horizontal gene transfer and point mutations
68 (10, 11).

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69 Feedlots in North America have traditionally administered antimicrobials to cattle to
70 prevent and treat disease (12). This includes classes of antimicrobials that are also used in human
71 medicine such as β-lactams, fluoroquinolones, macrolides, and tetracyclines (13, 14). However,
72 there is concern that the use of antimicrobials in food-producing animals selects for
73 antimicrobial-resistant bacteria that may be disseminated to humans through food and the
74 environment (15). Resistant strains of *E. faecium* isolated from meat have colonized the human
75 GI tract in challenge experiments (16) and transfer of the tetracycline resistance gene, *tet*(M)
76 from an *E. faecium* strain of meat origin to human clinical enterococci isolates has been
77 demonstrated *in vitro* (17). The culturability and ubiquity of *Enterococcus* spp. in cattle make
78 them ideal for monitoring antimicrobial resistance (AMR) in beef processing facilities and retail
79 products.

80 Therefore, in this study we isolated enterococci from samples taken from a commercial
81 beef processing facility over a nineteen-month period and from retail ground beef. The
82 antimicrobial susceptibility of selected *E. faecalis* and *E. faecium* isolates was determined and a
83 subset of these isolates were further characterized using whole genome sequencing. These
84 sequenced genomes were also compared with publicly available *E. faecalis* and *E. faecium*
85 genomes from different sources.

86 **RESULTS**

87 ***Enterococcus* spp. distribution and prevalence**

88 Ten different *Enterococcus* species were isolated from swabs and ground beef samples
89 with *E. faecalis*, *Enterococcus hirae*, and *E. faecium* most frequently recovered (Table 1). Within
90 the beef processing facility, the carcasses after hide removal and the ground beef yielded the

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91 greatest number of samples positive for enterococci. *E. faecalis* was the only species from all
92 five sampling locations.

93 **Antimicrobial susceptibility and detection of antimicrobial resistance genes**

94 Antimicrobial susceptibility testing was done on 120 *E. faecalis* and 9 *E. faecium* isolates
95 using 16 different antimicrobials (Table S1). Nearly all *E. faecalis* isolated on non-selective
96 media were resistance was to lincomycin (97.4%) and quinupristin-dalfopristin (92.8%) (Table
97 2). Phenotypic resistance to ciprofloxacin (10.8%), erythromycin (9.0%), and tetracycline
98 (28.8%) was also noted in several *E. faecalis* isolates. Although there were fewer *E. faecium*
99 isolates available for testing, resistance phenotypes were similar to *E. faecalis* with the exception
100 of ciprofloxacin resistance, which was not observed in any of the *E. faecium* strains. Two *E.*
101 *faecalis* isolates (H11 and H22) from the hide removal samples were resistant to nine
102 antimicrobials and one (G69E) from ground beef from the processing plant was resistant to six.
103 Only one *Enterococcus* isolate was susceptible to all 16 antimicrobials tested; however, no
104 resistance was recorded for linezolid, penicillin, or vancomycin for any of the isolates.

105 Among the 119 *E. faecalis* and 9 *E. faecium* isolates from selective and non-selective
106 media displaying phenotypic resistance to at least one antimicrobial, there were 21 unique AMR
107 profiles (Table S2). The most common AMR profiles included resistance to quinupristin-
108 dalfopristin and lincomycin (52.3%; 67) and quinupristin-dalfopristin, lincomycin, and
109 tetracycline (20.3%; 26). The *E. faecalis* and *E. faecium* isolates were also screened for the
110 presence of *erm*(B), *msrC*, *tet*(B), *tet*(C), *tet*(L), *tet*(M), *vanA*, *vanB*, and *vanC1* via PCR. The
111 *tet*(M) (26.5%) and *erm*(B) (7.7%) genes were detected most frequently in *E. faecalis* and *msrC*
112 (75.0%) and *erm*(B) (16.7%) in *E. faecium*. None of the *van* genes or *tet*(C) were found among
113 these isolates.

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114 **Genome analysis**

115 The assembly statistics for the 47 *E. faecalis* and 8 *E. faecium* genomes sequenced are
116 reported in Holman et al. (18) and Table S3. The size of the *E. faecalis* and *E. faecium* genomes
117 ranged from 2,647,103 to 3,246,301 bp and 2,507,908 to 2,761,265 bp, respectively.

118 **Antimicrobial resistance genes within genome assemblies**

119 We screened the *E. faecalis* and *E. faecium* assemblies for antimicrobial resistance genes
120 (ARGs) using the CARD RGI (Comprehensive Antibiotic Resistance Database Resistance Gene
121 Identifier) and identified 15 different ARGs conferring resistance to 8 different antimicrobial
122 classes. Similar to the PCR-based screening of select ARGs, *tet*(M) (31.9%) and *erm*(B) (8.5%)
123 were found most often within the *E. faecalis* genomes (Table 3). The genes *efrA*, *efrB*, *emeA,* and
124 *lsa*(A) which encode for multidrug efflux pumps (19, 20) were identified in all *E. faecalis*
125 genomes as was *dfrE*, a dihydrofolate reductase gene conferring resistance to
126 diaminopyrimidine. All sequenced *E. faecium* genomes carried the *aac(6')-Ii* and *msrC* genes
127 conferring resistance to aminoglycosides and macrolides-streptogramin B, respectively. The
128 *efmA* gene which encodes a multidrug efflux pump (21) was found in all but one of the *E.*
129 *faecium* genomes. The *aac(6')-Ii*, *efmA*, and *msrC* genes are considered to be intrinsic within *E.*
130 *faecium* (10). One *E. faecalis* strain (H11) that had been isolated from a hide prior to washing
131 carried 10 additional ARGs: *aac(6')-Ie-aph(2'')-Ia*, *aad(6)*, *ant(6)-Ia*, *aph(3')-IIIa*, *catA8*,
132 *erm*(B), *lsaE*, *sat4*, and *tet*(M). A different *E. faecalis* strain (H22) from the hides had seven
133 additional ARGs: *aad(6)*, *ant(6)-Ia*, *aph(3')-IIIa*, *lsaE*, *sat4*, and *tet*(M). These two isolates were
134 phenotypically resistant to nine different antimicrobials and had the same multi-locus sequence
135 typing (MLST) profile but were collected three months apart. The only other isolate with more
136 than two additional ARGs, *E. faecalis* H96E, was also collected from hides.

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137 Three *E. faecalis* (H11, H22, and H96E) and two *E. faecium* (H112E and H134E) isolates
138 with multidrug resistance profiles of interest were examined further to determine the genetic
139 context of the ARGs detected. All five multidrug-resistant strains contained an insertion
140 sequence harboring *tet*(M) (Fig. 1A) that had high sequence similarity (>80% identity and >70%
141 coverage when aligned using *E. faecium* H134E) to integrative and conjugative elements found
142 in *Streptococcus suis* (ICESsu05SC260; GenBank KX077888.1, ICESsuJH1308-2; GenBank
143 KX077884.1). Alignment of this region in all five isolates showed 85% pairwise identity and
144 revealed two variants with similarity in gene arrangements within *E. faecalis* H11, *E. faecalis*
145 H22, and *E. faecium* H112E and between *E. faecium* H134E and *E. faecalis* H96E. Differences
146 between the variants occurred on the left flank and included genes associated with integration
147 and the presence of *tet*(L) [designated *tet*(45) by the CARD RGI] adjacent to *tet*(M) in H96E and
148 H143E but not in H11, H22, and H112E. Despite complementarity, there were a significant
149 number of point mutations in this region between H11, H22, and H112E (88% pairwise identify)
150 that could reflect differences in the residence time of this gene region within each strain.

151 In *E. faecalis* H96E, approximately 60 kb upstream of *tet*(M), *erm*(B) was found
152 adjacent to a tetronasin resistance gene, a *tet*(R) gene, a transposase, a toxin-antitoxin system,
153 and other genes associated with transcriptional regulation (Fig. 1B). The *erm*(B) gene was also
154 present in *E. faecalis* H11 but was assembled as a single gene contig and therefore did not
155 provide information about its location within the genome. The *lsa*(E) gene in *E. faecalis* H11 and
156 H22 was found on contigs with identical gene arrangements that were truncated at the same
157 location on the left and right flank (Fig. 1C). In addition to *lsa*(E), these contigs also contained
158 an unnamed streptomycin 3''-adenylyltransferase and a lincosamide and streptogramin A
159 transport system ATP-binding/permease gene. The *E. faecalis* H11 and H22 assemblies also had

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160 contigs carrying the *aad*(6), *sat4*, *aph(3')-IIIa*, and *ant(6)-Ia* genes. Based on alignment against
161 multiple *Enterococcus* strains in NCBI, the *sat4* gene-containing contig was adjacent on the
162 chromosome to the contig carrying *lsa*(E), with the streptomycin 3''-adenylyltransferase and
163 *aad*(6) genes next to each other. As with other ARG regions found in these isolates, strong
164 pairwise identity was observed between parts of these contigs and similar cassettes found in
165 *Staphylococcus aureus* strains (*S. aureus* BA01611; RefSeq NC_007795.1, *S. aureus* MRSA_S3;
166 RefSeq NC_007795.1).

167 The aminoglycoside resistance genes *aac(6')-Ie-aph(2'')-Ia* and *ant(6)-Ia* were found
168 adjacent to one another, comprising a single contig in strain H11 (Fig. 1.D). This couplet of
169 ARGs is present in many *E. faecium* and *E. faecalis* strains in NCBI, but can also be found in
170 *Staphylococcus* spp., *Clostridium* spp., and *Campylobacter coli* strains. *E. faecium* H112E
171 contained a gene region harboring the oxazolidinone resistance gene *optrA* in close proximity to
172 the macrolide resistance gene *erm*(A), *ant(9)-Ia (*aminoglycoside resistance), and *xerC*, a
173 tyrosine recombinase gene (Fig. 1E). This gene region aligned with complete coverage and
174 greater than 99% identity to both a plasmid in *E. faecalis* (GenBank CP042214.1) and an *optrA*
175 gene cluster in *E. faecium* (GenBank MK251151.1) suggesting that this gene array could have
176 originally been a plasmid that integrated into the chromosome of *E. faecium* H112E. Other
177 ARGs present that either assembled into single gene contigs or gene regions lacking other ARGs
178 were the lincosamide resistance gene *lunG* in *E. faecalis* H96E, the chloramphenicol resistance
179 gene *catA*, and *msrC* in *E. faecium* H134E and H112E.

180 **Virulence genes**

181 Genome assemblies were also screened for virulence genes using the VirulenceFinder
182 *Enterococcus* database. The virulence genes *ace* (collagen adhesin), *camE*, *cCF10*, *cOB1* (sex

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183 pheromones), *ebpA*, *ebpB*, *ebpC* (pili proteins), *efaAfs* (adhesion), *elrA* (enterococcal leucine rich
184 protein A), *srtA* (sortase), *tpx* (thiol peroxidase) were found in all *E. faecalis* genomes (Table
185 S4). The gelatinase-encoding *gelE* and hyaluronidase genes *hylA* and *hylB* were also detected in
186 74.5%, 68.8%, and 83.0% of *E. faecalis* genomes, respectively. Only two *E. faecalis* genomes
187 carried the cytolysin genes *cylABLM* but notably these were also the strains that had the greatest
188 number of ARGs, H11 and H22. The *efaAfm* gene, which encodes a cell wall adhesin, was found
189 in all eight *E. faecium* assemblies. The *acm* gene (collagen-binding protein) was the only other
190 virulence gene detected in the *E. faecium* genomes (75%).

191 **Phylogeny of enterococcal strains**

192 Phylogenetic relationships among the 47 *E. faecalis* and 8 *E. faecium* strains and several
193 publicly available *E. faecalis* and *E. faecium* genomes were determined using the core genes
194 within each species. These additional *E. faecalis* and *E. faecium* strains included all publicly
195 available isolates from ground beef and several randomly selected human and cattle fecal isolates
196 also from Alberta (22). The core genome of *E. faecalis* contained 1,325 genes and the pan-
197 genome 9,558. Among the 27 *E. faecium* genomes included for analysis, there were 1,417 genes
198 in the core genome and 7,848 in the pan-genome.

199 *E. faecalis* strains clustered by MLST type (Fig. 2). Interestingly, certain *E. faecalis*
200 strains that had been collected from retail ground beef in the United States had a MLST profile
201 (ST192, ST228, and ST260) that was shared with strains isolated from the conveyor belt,
202 carcasses after final washing, and retail ground beef in the present study. Six of the *E. faecalis*
203 isolates (G92, G127E, G149, H4, W97, and W133) had the same MLST profile as one of the
204 Alberta human isolates (HC_NS0077; leg wound). However, it should be noted that this human
205 isolate carried *tet*(M) and an additional virulence gene which was absent from the six isolates.

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206 *E. faecium* isolates also clustered by MLST (Fig. 3). Three *E. faecium* isolates from retail
207 ground beef along with two isolates from the post-wash carcasses and one from US ground beef
208 had the same MLST (ST76). Unlike the *E. faecalis* genomes, there also appeared to be two
209 distinct clades of *E. faecium* with the two hide isolates (H134E and H112E) in a separate clade
210 from the other *E. faecium* isolates examined.

211 **Discussion**

212 Antimicrobial resistance continues to be a serious public health threat and there are
213 concerns that antimicrobial-resistant bacteria in food-producing animals may be transferred to
214 humans through the food production system. In this study we used culturing and whole genome
215 sequencing to monitor AMR and enterococci distribution in beef production from slaughter
216 through to the retail sector over a nineteen-month period. Although 10 different *Enterococcus*
217 spp. were isolated at least once during the study, only *E. faecalis* was found in all sampling
218 locations. This is consistent with previous surveys that sampled from beef plants (4) or retail
219 ground beef (5). *E. hirae* was isolated most frequently from post-hide removal swabs, which was
220 expected given that *E. hirae* has been reported to be the most prevalent *Enterococcus* spp. in
221 cattle feces (2, 22, 24) and there is greater likelihood of contamination from feces at the hide
222 removal step (25).

223 The number of enterococci-positive samples recovered from the carcass post-washing
224 and the conveyor belt area was substantially lower than in any other sample type. Carcasses are
225 subjected to washing with hot water and spraying with organic acids after hide removal which
226 reduces the microbial load on the carcasses. The proportion of enterococci isolated from the
227 conveyor belts was lower than an earlier study at the same plant (10.7% vs. 48%) (4). This may
228 represent improvements in sanitation within the conveyor area or possibly variation in the

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229 prevalence of enterococci. However, 82.7% of the ground beef produced within the plant was
230 positive for *Enterococcus* spp., most of which were *E. faecalis,* suggesting that the conveyor area
231 is not a reflection of the prevalence of enterococci in the ground beef produced. Enterococci
232 were also isolated from the majority of ground beef samples taken from retail stores in Alberta
233 which was similar to previous surveys of enterococci in retail ground beef in Alberta (4, 26) and
234 the United States (5).

235 We subjected 120 *E. faecalis* and 9 *E. faecium* isolates to antimicrobial susceptibility
236 testing due to their relevance to human health. Of the antimicrobials classified by the World
237 Health Organization (WHO) as critically important in human medicine (27), infrequent
238 resistance to ciprofloxacin, daptomycin, erythromycin, gentamicin, kanamycin, and tigecycline
239 was noted. None of the isolates were resistant to vancomycin or linezolid, antimicrobials often
240 used to treat VRE strains (28). Resistance to lincomycin and quinupristin-dalfopristin is intrinsic
241 in *E. faecalis* and mediated by the chromosomally-encoded *lsa*(A) gene (29), thus explaining the
242 widespread resistance of *E. faecalis* to these antimicrobials. Tetracycline resistance was observed
243 in 30% of *E. faecalis* and 33.3% of *E. faecium* isolates, which may have been due to the *tet*(M)
244 gene which was detected in 83.3% of tetracycline-resistant *E. faecalis* isolates and was absent in
245 tetracycline-susceptible ones. Feedlot cattle in Western Canada have historically received
246 tetracyclines such as chlortetracycline and oxytetracycline in feed or via injection for treatment
247 and prevention of disease, possibly accounting for the prevalence of tetracycline resistance noted
248 here (13).

249 Ionophores are one of the most widely used classes of antimicrobials in livestock
250 production. Because they are only employed in veterinary medicine it is assumed that their use
251 does not impact human health (30). As a potential human pathogen that inhabits the

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252 gastrointestinal tract of food-producing animals, several studies have examined ionophore
253 resistance in *Enterococcus* spp. but reported little or no concern for its development (31). If any
254 degree of resistance was observed it was attributed to thickening of the cell wall, or glycocalyx;
255 traits that were considered to be genetically unstable and reversible upon removal of selective
256 pressure (32). Recently, enterococci isolated from various locations around the world and from
257 both humans and animals, contained both the narasin gene which encodes for ionophore-
258 resistance and the *vanA* gene, raising the possibility that ionophore use may co-select for
259 vancomycin resistance in these strains (30). The existence of an isolate harboring both *erm*(B)
260 and a tetronasin resistance gene in our study merits further work to investigate possible linkages
261 between the use of in-feed ionophores and macrolide resistance.

262 A large portion of the ARG cassettes examined here are also found in *Streptococcus*,
263 *Staphylococcus* and *Campylobacter* spp. in NCBI. Future research that examines the rates of
264 prevalence and transmissibility of these mobile regions between and amongst these species
265 would be of considerable value in limiting the spread of AMR in bacteria of importance in
266 human disease. Several of the *E. faecalis* and *E. faecium* isolates from the post-washed carcasses,
267 conveyor belt area, and ground beef were genetically very similar to publicly available isolates
268 from ground beef in the United States, suggesting that these particular strains are well-adapted to
269 the beef packaging environment.

270 In summary, longitudinal sampling from a commercial beef packaging facility revealed
271 the presence of *E. faecalis* throughout the production environment with the greatest prevalence in
272 ground beef produced in the plant. Other *Enterococcus* spp. were isolated infrequently or as with
273 *E. hirae*, confined to the carcasses post-hide removal and ground beef in the facility. Among *E.*
274 *faecalis* isolates, the most frequently observed non-intrinsic phenotypic antimicrobial resistance

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275 was to tetracycline, which was likely mediated through the *tet*(M) gene. Several multidrug-
276 resistant isolates were recovered including two *E. faecalis* from hides which were resistant to
277 nine different antimicrobials and carried a number of ARGs on potentially mobile elements.
278 However, the risk that such strains found on the hides may pose to the food production system is
279 unknown as they were not isolated in the downstream processing environment.

280 **Materials and methods**

281 **Sampling and isolation of *Enterococcus* spp.**

282 Samples were collected a total of 15 times from July 2014 through February 2016 from a
283 commercial beef processing facility in Alberta, Canada that processed more than 3,000 carcasses
284 per day. During each visit 10 samples were obtained from each of four different areas within the
285 plant: carcasses after hide removal (H), carcasses after final washing (W), conveyer belts (C),
286 and ground beef made in the plant (G). A 2 cm x 2 cm gauze swab was used to sample a
287 randomly selected 10 cm x 10 cm area on the surface of the carcasses and conveyor belts. In
288 total, 150 samples were obtained from each sample type or location. During the same time
289 period, 60 samples of retail ground beef (R) were collected from various retail locations in
290 Alberta. The exact origin of these retail ground beef samples was unknown. All samples were
291 transported to the lab on ice and immediately processed. The swabs and 25 g of each ground
292 product and retail ground beef sample were transferred to a stomacher bag for homogenization
293 and pre-enrichment with 10 ml (swabs) or 225 ml (ground product/beef) of buffered peptone
294 water. These samples were then stomached at 260 rpm for 2 min in a Stomacher 400 Circulator
295 (Seward, Norfolk, UK) and incubated overnight at 37°C.

296 One milliliter of this mixture was then added to 9 ml of Enterococcosel broth (BD,
297 Mississauga, Ontario, Canada) with or without 8 μg ml^{-1} erythromycin (Sigma Aldrich Canada,

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298 Oakville, ON, USA) and incubated overnight at 37°C for the enrichment of enterococci.
299 Erythromycin was chosen since macrolides are important in human and veterinary medicine and
300 enterococci are not intrinsically resistant to this antimicrobial. Enterococcosel broth tubes
301 displaying evidence of esculin hydrolysis (black) were streaked onto Enterococcosel agar with
302 and without 8 μg ml^{-1} erythromycin and incubated at 37°C. After 48 h the plates were examined
303 for colonies with black zones (esculin hydrolysis) and three colonies from each plate were re-
304 streaked onto Enterococcosel agar and incubated for 48 h at 37°C. One positive colony from
305 each agar plate was then transferred to 1 ml of brain heart infusion (Dalynn Biologicals, Calgary,
306 AB, Canada) containing 15% glycerol and frozen at -80°C. Confirmation and species
307 identification of presumptive enterococci isolates was done via PCR with the Ent-ES-211-233-F
308 and Ent-EL-74-95-R primers (33) to amplify the *groES-EL* spacer region as previously described
309 (2). *Enterococcus hirae* were identified using primers mur2h-F 5'-
310 TATGGATACACTCGAATATCTT-3' and 5'-ATTATTCCATTCGATTAACTGC-3' to target
311 the muramidase (*mur-2*) gene of *E. hirae* as per Zaheer et al. (22). The *groES-EL* amplicon from
312 non-*E. hirae* isolates was sequenced on an ABI Prism 3130xl Genetic Analyzer (Thermo Fisher
313 Scientific Inc., Mississauga, ON, Canada) to differentiate *Enterococcus* spp.

314 **Antimicrobial resistance screening of enterococci isolates**

315 Due to their relevance to human health, isolates with a *groES-EL* spacer region that was
316 100% identical to *E. faecalis* or *E. faecium* were screened for antimicrobial resistance genes
317 (ARGs) and antimicrobial sensitivity. Broth microdilution with the Sensititre NARMS Gram-
318 positive CMV3AGPF AST plate (Trek Diagnostics, Independence, OH, USA) was used to
319 determine the susceptibility of 120 *E. faecalis* and 9 *E. faecium* isolates to sixteen different
320 antimicrobials. For antimicrobials in the panel, the Clinical and Laboratory Standards Institute

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321 (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) minimum
322 inhibitory concentration (MIC) breakpoints for *Enterococcus* spp. were used to interpret the
323 results. These isolates were also screened via PCR for the presence of the ARGs *erm*(B), *msrC*,
324 *tet*(B), *tet*(C), *tet*(L), *tet*(M), *vanA*, *vanB*, and *vanC1* as described in Beukers et al. (2) (Table S5).

325 **Sequencing of selected *Enterococcus faecalis* and *Enterococcus faecalis* isolates**

326 Forty-seven *E. faecalis* and eight *E. faecium* isolates were selected for whole genome
327 sequencing based on their AMR profiles and sample origin. Briefly, the isolates were re-cultured
328 from the frozen glycerol on BEA and incubated for 24 h at 37°C to obtain isolated colonies with
329 typical morphology and colour. A single colony was then streaked onto BHI agar (Dalynn
330 Biologicals), grown overnight at 37°C, and colonies from this plate were suspended in 10 mM
331 Tris-1mM EDTA (TE) (pH 8.0) buffer to obtain an OD_{600} of 2.0 (2×10^9 cells ml^{-1}). One
332 milliliter of this suspension was pelleted via centrifugation at 14,000 x g for 2 min. Genomic
333 DNA was extracted from the pellet using the DNeasy Blood and Tissue kit (Qiagen,
334 Mississauga, Ontario, Canada) with the modification that cells were incubated with agitation
335 (150 rpm) for 45 min at 37°C in 280 µl of lysis buffer (20 mM Tris-HCl [pH 8.0], 2 mM sodium
336 EDTA, 1.2% Triton X-100 and 20 mg ml^{-1} lysozyme) (Sigma Aldrich Canada) prior to the
337 addition of proteinase K and 5 µl of 100 mg ml^{-1} RNase A (Qiagen). The DNA concentration
338 was determined using a Qubit fluorometer (Thermo Fisher Scientific, Mississauga, ON, Canada).
339 The Nextera XT DNA Library Preparation kit (Illumina Inc., San Diego, CA, USA) was used to
340 prepare sequencing libraries that were sequenced on a MiSeq instrument (Illumina Inc., San
341 Diego, CA, USA) with the MiSeq Reagent kit v3 (Illumina Inc.; 600 cycles) or on a NovaSeq
342 6000 machine (Illumina Inc.) with a SP flowcell (300 cycles).

343 **Genomic analysis of *Enterococcus faecalis* and *Enterococcus faecalis* isolates.**

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344 Trimmomatic v. 0.39 (34) was used to remove sequencing adapters, reads with a quality
345 score of less than 15 over a 4-bp sliding window, and reads that were less than 50 bp in length.
346 Genomes were assembled with SPAdes v. 3.15.1 (35) in "isolate mode" and the quality of the
347 assemblies was assessed with QUAST v. 5.0.2 (36). Potential contamination within each
348 assembly was determined using Kraken 2 v. 2.1.1 and the minikraken2 database v. 2 (37) as
349 well as CheckM v. 1.1.3 (38). GTDB-tk v. 1.3.0 (39) was also used to confirm the taxonomic
350 assignments of the assemblies and Prokka v. 1.14.6 (40) was used to annotate the assemblies.
351 Determination of MLST was done on the assembled genomes using the *E. faecalis*
352 (https://pubmlst.org/efaecalis) and *E. faecium* (https://pubmlst.org/efaecium/) MLST databases
353 (41, 42).

354 The accessory, core, and pan-genome of the *E. faecalis* and *E. faecium* genomes were
355 identified using Roary v. 3.13.0 (43) with a BLASTp identity cut-off of ≥95%. The core genome
356 is defined as genes present in ≥ 99% of genomes. The core genes for both species were aligned
357 in Roary using MAFFT v. 7.475 (44) and a maximum likelihood phylogenetic tree was inferred
358 from this alignment using RAxML v. 8.2.12 (45) and viewed with ggtree v. 2.4.1 (46) in R
359 3.6.1.. Several publicly available *E. faecalis* and *E. faecium* assemblies from various isolation
360 sources, including from humans and cattle in Alberta, were also included in the core and pan-
361 genome analysis as listed in Table S6. The genome assemblies were screened for virulence genes
362 using the VirulenceFinder 2.0 database (47) and BLASTn (≥90% identity) and for ARGs using
363 the CARD v. 3.0.9 (48) Resistance Gene Identifier (RGI). The depicted gene regions containing
364 ARGs were constructed and validated using contig alignments in Geneious v. 11.0.9. BLAST
365 was used to identify highly similar regions with >80% pairwise identity in bacterial strains
366 present in NCBI.

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376 **References**

377 1. Boehm AB, Sassoubre LM. 2014. Enterococci as indicators of environmental fecal
378 contamination, Enterococci: From Commensals to Leading Causes of Drug Resistant
379 Infection [Internet]. Massachusetts Eye and Ear Infirmary.
380 2. Beukers AG, Zaheer R, Cook SR, Stanford K, Chaves AV, Ward MP, McAllister TA.
381 2015. Effect of in-feed administration and withdrawal of tylosin phosphate on antibiotic
382 resistance in enterococci isolated from feedlot steers. Front Microbiol 6:483.
383 https://doi.org/10.3389/fmicb.2015.00483.
384 3. Farnleitner AH, Ryzinska-Paier G, Reischer GH, Burtscher MM, Knetsch S, Kirschner
385 AK, Dirnbock T, Kuschnig G, Mach RL, Sommer R. 2010. *Escherichia coli* and
386 enterococci are sensitive and reliable indicators for human, livestock and wildlife faecal
387 pollution in alpine mountainous water resources. J Appl Microbiol 109:1599-608.
388 https://doi.org/10.1111/j.1365-2672.2010.04788.x.

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389 4. Aslam M, Diarra MS, Service C, Rempel H. 2010. Characterization of antimicrobial
390 resistance in *Enterococcus* spp. recovered from a commercial beef processing plant.
391 Foodborne Pathog Dis 7:235-41. https://doi.org/10.1089/fpd.2009.0380.
392 5. Tyson GH, Nyirabahizi E, Crarey E, Kabera C, Lam C, Rice-Trujillo C, McDermott PF,
393 Tate H. 2018. Prevalence and Antimicrobial Resistance of Enterococci Isolated from
394 Retail Meats in the United States, 2002 to 2014. Appl Environ Microbiol 84:e01902-17.
395 https://doi.org/10.1128/AEM.01902-17.
396 6. Hayes JR, English LL, Carter PJ, Proescholdt T, Lee KY, Wagner DD, White DG. 2003.
397 Prevalence and Antimicrobial Resistance of *Enterococcus* Species Isolated from Retail
398 Meats. Appl Environ Microbiol 69:7153-60. https://doi.org/10.1128/aem.69.12.7153-
399 7160.2003.
400 7. Schmidt JW VA, Doster E, Thomas K, Weinroth MD, Parker J, Hanes A, Geornaras I,
401 Morley PS, Belk KE, Wheeler TL, Arthur TM. 2020. Antimicrobial Resistance in U.S.
402 Retail Ground Beef with and without Label Claims Regarding Antibiotic Use. J Food
403 Prot 84:827-842. https://doi.org/0.4315/JFP-20-376.
404 8. Simner PJ, Adam H, Baxter M, McCracken M, Golding G, Karlowsky JA, Nichol K,
405 Lagace-Wiens P, Gilmour MW, Canadian Antimicrobial Resistance A, Hoban DJ, Zhanel
406 GG. 2015. Epidemiology of vancomycin-resistant enterococci in Canadian hospitals
407 (CANWARD study, 2007 to 2013). Antimicrob Agents Chemother 59:4315-7.
408 https://doi.org/10.1128/AAC.00384-15.
409 9. Higuita NIA, Huycke MM. 2014. Enterococcal disease, epidemiology, and implications
410 for treatment, Enterococci: From commensals to leading causes of drug resistant infection
411 [Internet]. Massachusetts Eye and Ear Infirmary.

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412 10. Hollenbeck BL, Rice LB. 2012. Intrinsic and acquired resistance mechanisms in
413 enterococcus. Virulence 3:421-33. https://doi.org/10.4161/viru.21282.
414 11. Arias CA, Murray BE. 2012. The rise of the *Enterococcus*: beyond vancomycin
415 resistance. Nat Rev Microbiol 10:266-78. https://doi.org/10.1038/nrmicro2761.
416 12. Cameron A, McAllister TA. 2016. Antimicrobial usage and resistance in beef production.
417 J Anim Sci Biotechnol 7:68. https://doi.org/10.1186/s40104-016-0127-3.
418 13. Brault SA HS, Gow SP, Warr BN, Withell J, Song J, Williams CM, Otto SJG, Booker
419 CW, Morley PS. 2019. Antimicrobial Use on 36 Beef Feedlots in Western Canada: 2008-
420 2012. Front Vet Sci 6:329. https://doi.org/10.3389/fvets.2019.00329.
421 14. USDA. 2019. Antimicrobial Use and Stewardship on U.S. Feedlots, 2017.
422 https://www.aphis.usda.gov/animal_health/nahms/amr/downloads/amu-feedlots_1.pdf.
423 15. Van Boeckel TP BC, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teillant A,
424 Laxminarayan R. 2015. Global trends in antimicrobial use in food animals. Proc Natl
425 Acad Sci U S A 112:5649-5654. https://doi.org/10.1073/pnas.1503141112.
426 16. Sorensen TL, Blom M, Monnet DL, Frimodt-Moller N, Poulsen RL, Espersen F. 2001.
427 Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium*
428 from chicken and pork. N Engl J Med 345:1161-6.
429 https://doi.org/10.1056/NEJMoa010692.
430 17. Jahan M, Zhanel GG, Sparling R, Holley RA. 2015. Horizontal transfer of antibiotic
431 resistance from *Enterococcus faecium* of fermented meat origin to clinical isolates of *E.*
432 *faecium* and *Enterococcus faecalis*. Int J Food Microbiol 199:78-85.
433 https://doi.org/10.1016/j.ijfoodmicro.2015.01.013.

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434 18. Holman DB, Gzyl KE, Zaheer R, Jones TH, McAllister TA. 2019. Draft Genome
435 Sequences of 43 *Enterococcus faecalis* and *Enterococcus faecium* Isolates from a
436 Commercial Beef Processing Plant and Retail Ground Beef. Microbiol Resour Announc
437 8:e00974-19. https://doi.org/10.1128/MRA.00974-19.

438 19. Jonas BM MB, Weinstock GM. 2001. Characterization of *emeA*, a NorA homolog and
439 multidrug resistance efflux pump, in *Enterococcus faecalis*. Antimicrob Agents
440 Chemother 45:3574-3579. https://doi.org/10.1128/AAC.45.12.3574-3579.2001.

441 20. Lee EW, Huda MN, Kuroda T, Mizushima T, Tsuchiya T. 2003. EfrAB, an ABC
442 multidrug efflux pump in *Enterococcus faecalis*. Antimicrob Agents Chemother 47:3733-
443 8. https://doi.org/10.1128/aac.47.12.3733-3738.2003.

444 21. Nishioka T, Ogawa W, Kuroda T, Katsu T, Tsuchiya T. 2009. Gene cloning and
445 characterization of EfmA, a multidrug efflux pump, from *Enterococcus faecium*. Biol
446 Pharm Bull 32:483-8. https://doi.org/10.1248/bpb.32.483.

447 22. Zaheer R CS, Barbieri R, Goji N, Cameron A, Petkau A, Polo RO, Tymensen L, Stamm
448 C, Song J, Hannon S, Jones T, Church D, Booker CW, Amoako K, Van Domselaar G,
449 Read RR, McAllister TA. 2020. Surveillance of *Enterococcus* spp. reveals distinct
450 species and antimicrobial resistance diversity across a One-Health continuum. Sci Rep
451 10:1-16. https://doi.org/10.1038/s41598-020-61002-5.

452 23. Zhou W GS, Xu H, Zhang Z, Chen F, Shen H, Zhang C. 2019. Distribution of the *optrA*
453 gene in *Enterococcus* isolates at a tertiary care hospital in China. J Glob Antimicrob
454 Resist 17:180-186. https://doi.org/10.1016/j.jgar.2019.01.001.

455 24. Zaheer R, Cook SR, Klima CL, Stanford K, Alexander T, Topp E, Read RR, McAllister
456 TA. 2013. Effect of subtherapeutic vs. therapeutic administration of macrolides on

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.28.446255; this version posted May 29, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

457 antimicrobial resistance in *Mannheimia haemolytica* and enterococci isolated from beef
458 cattle. Front Microbiol 4:133. https://doi.org/10.3389/fmicb.2013.00133.
459 25. Fluckey WM LG, Warner RD, Echeverry A, Brashears MM. 2009. Diversity and
460 susceptibility of *Enterococcus* isolated from cattle before and after harvest. J Food Prot
461 72:766-774. https://doi.org/10.4315/0362-028x-72.4.766.
462 26. Aslam M, Diarra MS, Checkley S, Bohaychuk V, Masson L. 2012. Characterization of
463 antimicrobial resistance and virulence genes in *Enterococcus* spp. isolated from retail
464 meats in Alberta, Canada. Int J Food Microbiol 156:222-30.
465 https://doi.org/10.1016/j.ijfoodmicro.2012.03.026.
466 27. Organization WH. 2018. Critically important antimicrobials for human medicine: ranking
467 of antimicrobial agents for risk management of antimicrobial resistance due to non-
468 human use.
469 28. Balli EP, Venetis CA, Miyakis S. 2014. Systematic review and meta-analysis of linezolid
470 versus daptomycin for treatment of vancomycin-resistant enterococcal bacteremia.
471 Antimicrob Agents Chemother 58:734-9. https://doi.org/10.1128/AAC.01289-13.
472 29. Singh KV WG, Murray BE. 2002. An *Enterococcus faecalis* ABC homologue (Lsa) is
473 required for the resistance of this species to clindamycin and quinupristin-dalfopristin.
474 Antimicrob Agents Chemother 46:1845-1850. https://doi.org/10.1128/aac.46.6.1845-
475 1850.2002.
476 30. Wong A. 2019. Unknown Risk on the Farm: Does Agricultural Use of Ionophores
477 Contribute to the Burden of Antimicrobial Resistance? mSphere 4:e00433-19.
478 https://doi.org/10.1128/mSphere.00433-19.

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.28.446255; this version posted May 29, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

479 31. Nisbet DJ, Callaway TR, Edrington TS, Anderson RC, Poole TL. 2008. Effects of
480 ionophores on *Enterococcus faecalis* and *E. faecium* growth in pure and mixed ruminal
481 culture. Foodborne Pathog Dis 5:193-8. https://doi.org/10.1089/fpd.2007.0058.
482 32. Simjee S, Heffron AL, Pridmore A, Shryock TR. 2012. Reversible monensin adaptation
483 in *Enterococcus faecium*, *Enterococcus faecalis* and *Clostridium perfringens* of cattle
484 origin: potential impact on human food safety. J Antimicrob Chemother 67:2388-95.
485 https://doi.org/10.1093/jac/dks236.
486 33. Zaheer R YL, Church D, Topp E, Read RR, McAllister TA. 2012. High-throughput
487 species identification of enterococci using pyrosequencing. J Microbiol Methods 89:174-
488 178. https://doi.org/10.1016/j.mimet.2012.03.012.
489 34. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina
490 sequence data. Bioinformatics 30:2114-20.
491 https://doi.org/10.1093/bioinformatics/btu170.
492 35. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM,
493 Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G,
494 Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its
495 applications to single-cell sequencing. J Comput Biol 19:455-77.
496 https://doi.org/10.1089/cmb.2012.0021.
497 36. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for
498 genome assemblies. Bioinformatics 29:1072-5.
499 https://doi.org/10.1093/bioinformatics/btt086.
500 37. Wood DE, Lu J, Langmead B. 2019. Improved metagenomic analysis with Kraken 2.
501 Genome Biol 20:257. https://doi.org/10.1186/s13059-019-1891-0.

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.28.446255; this version posted May 29, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

502 38. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM:
503 assessing the quality of microbial genomes recovered from isolates, single cells, and
504 metagenomes. Genome Res 25:1043-55. https://doi.org/10.1101/gr.186072.114.
505 39. Chaumeil PA MA, Hugenholtz P, Parks DH. 2020. GTDB-Tk: a toolkit to classify
506 genomes with the Genome Taxonomy Database. Bioinformatics 36:1925–1927.
507 https://doi.org/10.1093/bioinformatics/btz848.
508 40. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics
509 30:2068-9. https://doi.org/10.1093/bioinformatics/btu153.
510 41. Ruiz-Garbajosa P, Bonten MJ, Robinson DA, Top J, Nallapareddy SR, Torres C, Coque
511 TM, Canton R, Baquero F, Murray BE, del Campo R, Willems RJ. 2006. Multilocus
512 sequence typing scheme for *Enterococcus faecalis* reveals hospital-adapted genetic
513 complexes in a background of high rates of recombination. J Clin Microbiol 44:2220-8.
514 https://doi.org/10.1128/JCM.02596-05.
515 42. Homan WL, Tribe D, Poznanski S, Li M, Hogg G, Spalburg E, Van Embden JD, Willems
516 RJ. 2002. Multilocus sequence typing scheme for *Enterococcus faecium*. J Clin Microbiol
517 40:1963-71. https://doi.org/10.1128/jcm.40.6.1963-1971.2002.
518 43. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MT, Fookes M, Falush D,
519 Keane JA, Parkhill J. 2015. Roary: rapid large-scale prokaryote pan genome analysis.
520 Bioinformatics 31:3691-3. https://doi.org/10.1093/bioinformatics/btv421.
521 44. Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7:
522 improvements in performance and usability. Mol Biol Evol 30:772-80.
523 https://doi.org/10.1093/molbev/mst010.

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.28.446255; this version posted May 29, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

524 45. Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis
525 of large phylogenies. Bioinformatics 30:1312-3.
526 https://doi.org/10.1093/bioinformatics/btu033.
527 46. Yu G, Smith DK, Zhu H, Guan Y, Lam TTYJMiE, Evolution. 2017. ggtree: an R
528 package for visualization and annotation of phylogenetic trees with their covariates and
529 other associated data. 8:28-36.
530 47. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM. 2014.
531 Real-time whole-genome sequencing for routine typing, surveillance, and outbreak
532 detection of verotoxigenic *Escherichia coli*. J Clin Microbiol 52:1501-10.
533 https://doi.org/10.1128/JCM.03617-13.
534 48. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, Huynh W,
535 Nguyen AV, Cheng AA, Liu S, Min SY, Miroshnichenko A, Tran HK, Werfalli RE,
536 Nasir JA, Oloni M, Speicher DJ, Florescu A, Singh B, Faltyn M, Hernandez-
537 Koutoucheva A, Sharma AN, Bordeleau E, Pawlowski AC, Zubyk HL, Dooley D,
538 Griffiths E, Maguire F, Winsor GL, Beiko RG, Brinkman FSL, Hsiao WWL, Domselaar
539 GV, McArthur AG. 2020. CARD 2020: antibiotic resistome surveillance with the
540 comprehensive antibiotic resistance database. Nucleic Acids Res 48:D517-D525.
541 https://doi.org/10.1093/nar/gkz935.

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547 **Figure Legends**

548 **Figure 1**. Location of antimicrobial resistance genes (ARGs) within indicated *Enterococcus*
549 *faecalis* and *Enterococcus faecium* strains. The ARGs are displayed in yellow, non-ARGs genes
550 are blue, and hypothetical proteins are colored grey.

551 **Figure 2**. Maximum likelihood phylogeny of 47 *Enterococcus faecalis* isolates from the current
552 study and selected publicly available *E. faecalis* genomes from cattle feces (n = 10), ground beef
553 (n = 7), and humans (n = 12). Phylogeny was inferred from the alignment of 1,325 core genes
554 using RAxML. Scale bar represents substitutions per nucleotide.

555 **Figure 3**. Maximum likelihood phylogeny of 8 *Enterococcus faecium* isolates and selected
556 publicly available *E. faecium* genomes from cattle feces (n = 5), ground beef (n = 7), and humans
557 (n = 7). Phylogeny was inferred from the alignment of 1,417 core genes using RAxML. Scale bar
558 represents substitutions per nucleotide.

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569 **Table 1**. Distribution and prevalence of *Enterococcus* spp. in swabs and samples from four
570 different locations in a beef processing facility (n = 150) and in retail ground beef (n = 60).
571 Values represent the number of positive swabs or samples and include isolates from both
572 selective (erythromycin) and non-selective media.

	Hide removal	**After final washing**	**Conveyor belt**	**Ground beef from processing facility**	**Ground beef from retail**	**Total**
E. faecalis	32	11	11	114	41	**209**
E. hirae	45	3	0	30	0	**78**
E. faecium	4	2	0	5	7	**18**
E. raffinosus	0	0	1	8	0	**9**
E. malodoratus	2	2	2	2	0	**8**
E. durans	8	0	0	0	0	**8**
E. gallinarum	1	0	2	0	1	**4**
E. casseliflavus	3	0	0	0	0	**3**
E. avium	0	0	0	0	1	**1**
E. mundtii	1	0	0	0	0	**1**
% Positive for *Enterococcus* spp.	51.3% (77)	12.0% (18)	10.7% (16)	82.7% (124)	81.7% (49)	

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576 **Table 2**. Antimicrobial susceptibility for *E. faecalis* (n = 111) isolated on non-selective media by
577 antimicrobial and isolation source. Values represent percentage of isolates that are resistant and
578 numbers in parentheses indicate total number of isolates. None of the isolates were resistant to
579 linezolid, nitrofurantoin, penicillin, tigecycline, or vancomycin.

Antimicrobial class	Antimicrobial	Species	After hide removal (H)	After final washing (W)	Conveyor belt (C)	Ground beef from processing facility (G)	Ground beef from retail (R)	Total
Aminoglycosides	GEN	*E. faecalis*	11.1% (2)	0	0	0	0	1.8% (2)
	KAN	*E. faecalis*	11.1% (2)	0	0	0	0	1.8% (2)
	STR	*E. faecalis*	11.1% (2)	0	0	0	0	1.8% (2)
Fluoroquinolones	CIP	*E. faecalis*	5.6% (1)	0	28.6% (2)	11.8% (4)	11.6% (5)	10.8% (12)
Lincosamides	LIN	*E. faecalis*	100% (18)	100% (9)	100% (7)	94.1% (32)	97.7% (42)	97.3% (108)
Lipopeptides	DAP	*E. faecalis*	0	0	0	5.9% (2)	0	1.8% (2)
Macrolides	ERY	*E. faecalis*	11.1% (2)	11.1% (1)	0	14.7% (5)	4.6% (2)	9.0% (10)
	TYL	*E. faecalis*	11.1% (2)	0	0	2.9% (1)	2.3% (1)	3.6% (4)
Phenicols	CHL	*E. faecalis*	11.1% (2)	0	0	0	0	1.8% (2)
Streptogramins	SYN	*E. faecalis*	94.4% (17)	77.7% (7)	100% (7)	94.1% (32)	93.0% (40)	92.8% (103)
Tetracyclines	TET	*E. faecalis*	11.1% (2)	11.1% (1)	14.3% (1)	50.0% (17)	25.6% (11)	28.8% (32)

580 NI: not isolated; CHL: chloramphenicol; CIP: ciprofloxacin; DAP: daptomycin; ERY:
581 erythromycin; GEN: gentamicin; KAN: kanamycin; LIN: lincomycin; STR: streptomycin; SYN:
582 quinupristin-dalfopristin; TET: tetracycline; TYL: tylosin.
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585 **Table 3**. Antimicrobial resistance genes identified in sequenced *Enterococcus faecalis* (n = 47)

586 and *Enterococcus faecium* (n = 8) genomes.

Gene	**Product**	**Target**	***E. faecalis***	***E. faecium***
aac(6')-Ii	Acetyltransferase	Aminoglycosides	0	100% (8)
ant(6)-Ia	Nucleotidyltransferase	Aminoglycosides	4.3% (2)	0
ant(9)-Ia	Nucleotidyltransferase	Aminoglycosides	0	12.5% (1)
aph(3')-IIIa	Phosphotransferase	Aminoglycosides	4.3% (2)	0
lnuG	Nucleotidyltransferase	Lincosamides	2.1% (1)	0
msrC	ABC transporter	Macrolides	0	100% (8)
erm(A)	23S rRNA methyltransferase	Macrolides	0	12.5% (1)
erm(B)	23S rRNA methyltransferase	Macrolides	8.5% (4)	12.5% (1)
optrA	ABC transporter	Oxazolidinones	0	12.5% (1)
lpsB	Intrinsic peptide antibiotic-resistant LPS	Peptides	2.1% (1)	0
catA8	Chloramphenicol acetyltransferase	Phenicols	2.1% (1)	0
lsa(E)	ABC transporter	Multiple drugs	4.3% (2)	0
sat4	Acetyltransferase	Streptothricins	4.3% (2)	0
tet(45)	Efflux protein	Tetracyclines	2.1% (1)	12.5% (1)
tet(M)	Ribosomal protection protein	Tetracyclines	31.9% (15)	37.5% (3)

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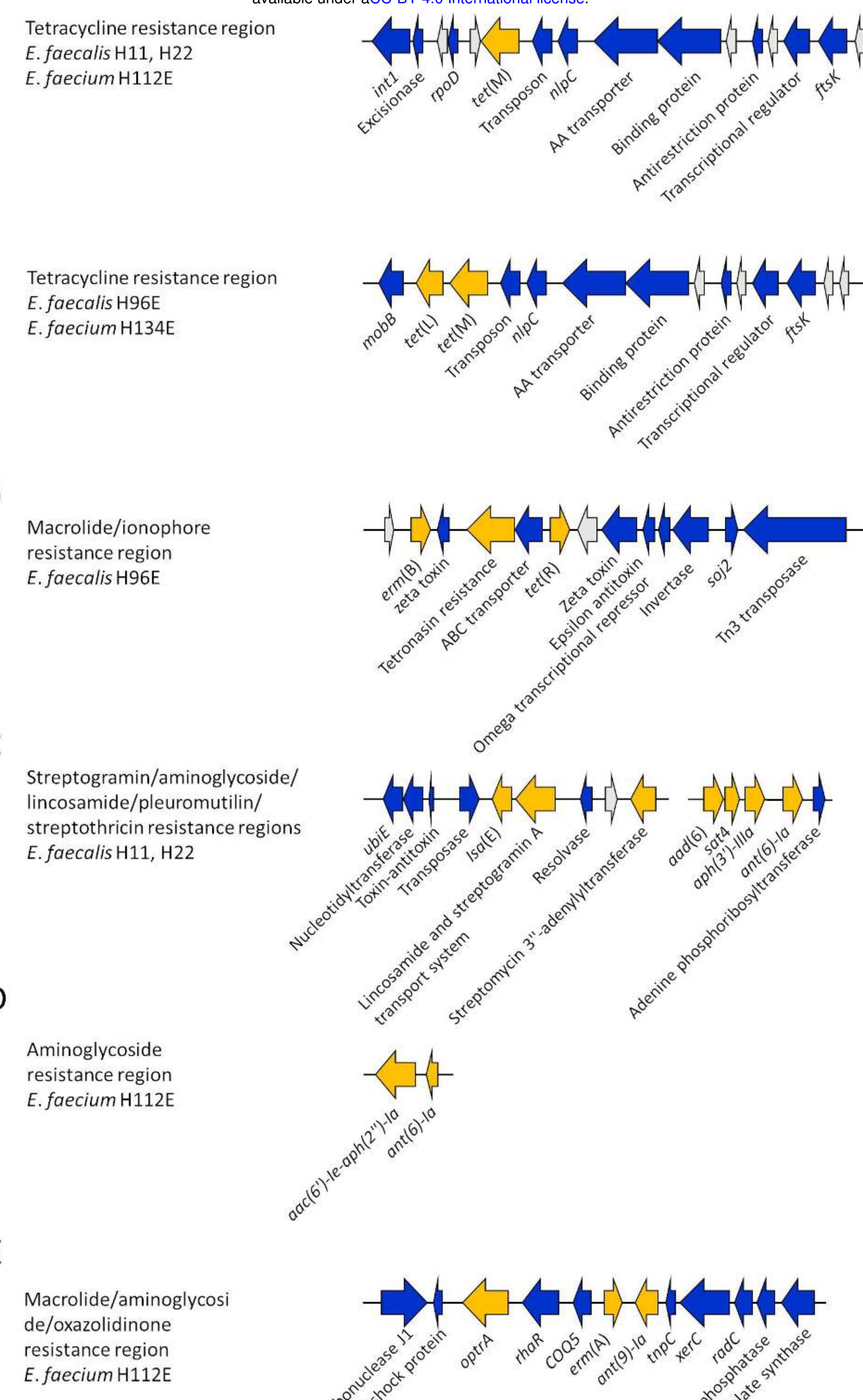

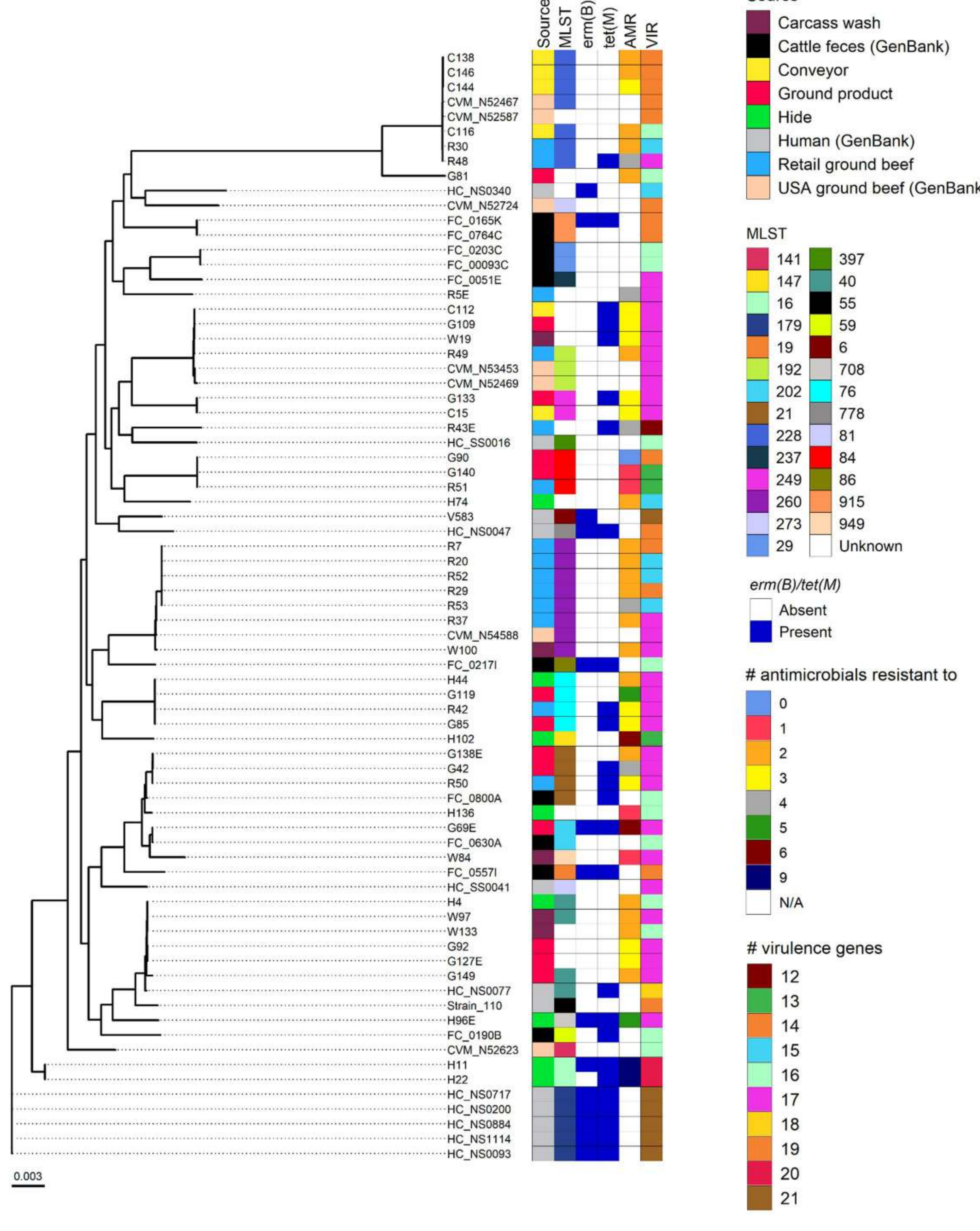
Source
MLST
erm(B)
tet(M)
AMR
VIR
C138
C146
C144
CVM_N52467
CVM_N52587
C116
R30
R48
G81
HC_NS0340
CVM_N52724
FC_0165K
FC_0764C
FC_0203C
FC_00093C
FC_0051E
R5E
C112
G109
W19
R49
CVM_N53453
CVM_N52469
G133
C15
R43E
HC_SS0016
G90
G140
R51
H74
V583
HC_NS0047
R7
R20
R52
R29
R53
R37
CVM_N54588
W100
FC_0217I
H44
G119
R42
G85
H102
G138E
G42
R50
FC_0800A
H136
G69E
FC_0630A
W84
FC_0557I
HC_SS0041
H4
W97
W133
G92
G127E
G149
HC_NS0077
Strain_110
H96E
FC_0190B
CVM_N52623
H11
H22
HC_NS0717
HC_NS0200
HC_NS0884
HC_NS1114
HC_NS0093
0.003
Source
Carcass wash
Cattle feces (GenBank)
Conveyor
Ground product
Hide
Human (GenBank)
Retail ground beef
USA ground beef (GenBank)
MLST
141
147
16
179
19
192
202
21
228
237
249
260
273
29
397
40
55
59
6
708
76
778
81
84
86
915
949
Unknown
erm(B)/tet(M)
Absent
Present
antimicrobials resistant to
0
1
2
3
4
5
6
9
N/A
virulence genes
12
13
14
15
16
17
18
19
20
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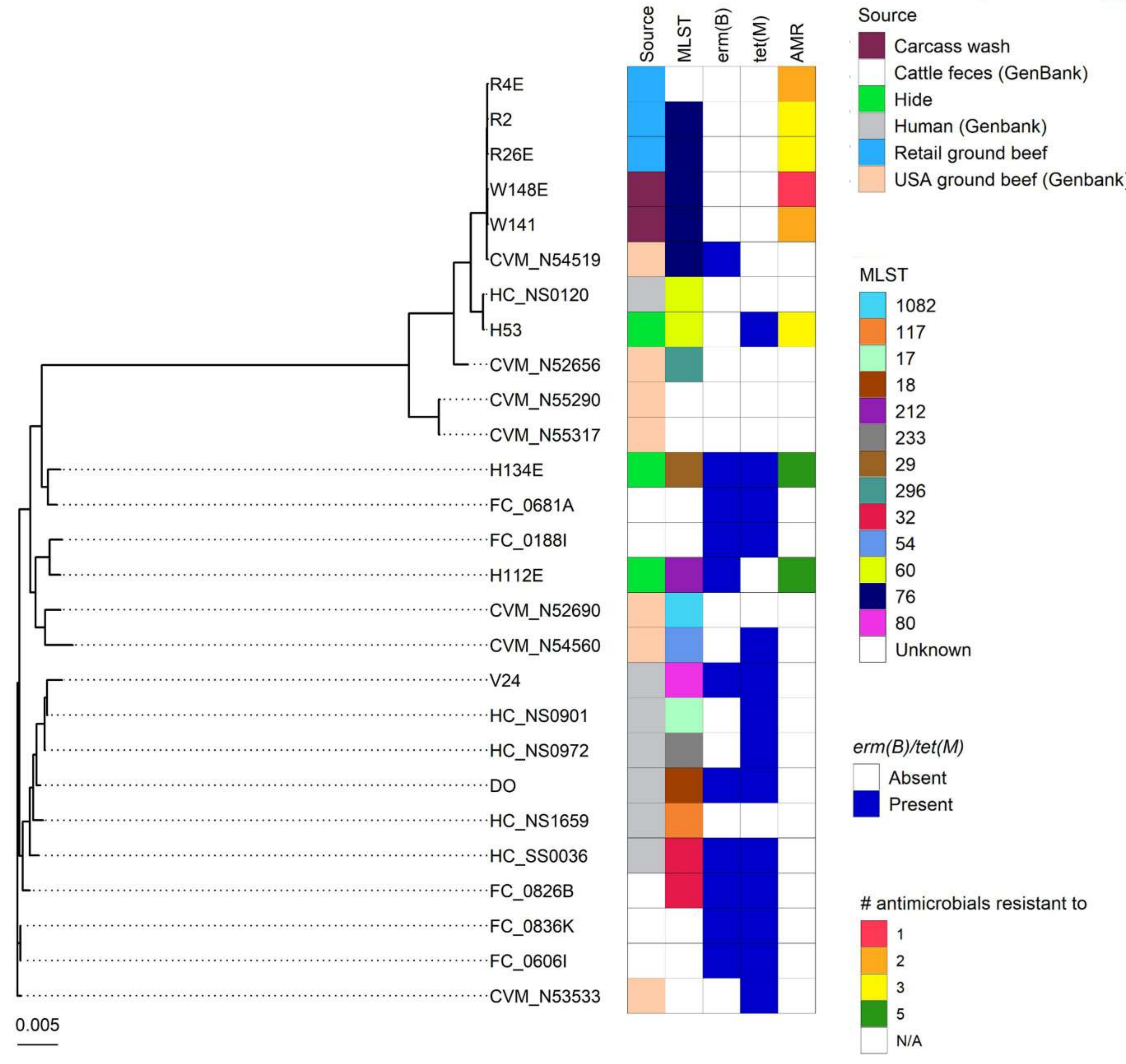
Source
MLST
erm(B)
tet(M)
AMR
R4E
R2
R26E
W148E
W141
CVM_N54519
HC_NS0120
H53
CVM_N52656
CVM_N55290
CVM_N55317
H134E
FC_0681A
FC_0188I
H112E
CVM_N52690
CVM_N54560
V24
HC_NS0901
HC_NS0972
DO
HC_NS1659
HC_SS0036
FC_0826B
FC_0836K
FC_0606I
CVM_N53533
0.005
Source
Carcass wash
Cattle feces (GenBank)
Hide
Human (Genbank)
Retail ground beef
USA ground beef (Genbank)
MLST
1082
117
17
18
212
233
29
296
32
54
60
76
80
Unknown
erm(B)/tet(M)
Absent
Present
antimicrobials resistant to
1
2
3
5
N/A